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6. AUTHOR(S) McFall-Ngai, Margaret				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Pacific Biomedical Research Center University of Hawaii 41 Ahui Street Honolulu, HI 96813			8. PERFORMING ORGANIZATION REPORT NUMBER	
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13. ABSTRACT (Maximum 200 words) The symbiosis between the Hawaiian sepiolid squid <i>Euprymna scolopes</i> and its marine luminous bacterial partner <i>Vibrio fischeri</i> was developed as a model system by which to study the process of recognition and specificity in the establishment of animal-bacterial associations. These early studies on this system revealed that complex, redundant systems ensure the fidelity of the relationship between these two partners. Using routine techniques of cell biology, biochemistry and molecular biology, we determined that specific receptor-ligand interactions are required during colonization, and that proteins thus far only associated with pathogenic associations are recruited into the function of modulating this cooperative symbiosis. In addition, comparative studies with different host and symbionts species, obtained from various locations around the globe, demonstrated that the squid-vibrio relationships have coevolved, and determined that the host-symbiont specificity is determined during the early recognition phases of the association.				
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FINAL REPORT

GRANT #: N00014-91-J-1357

PRINCIPAL INVESTIGATOR: Margaret J. McFall-Ngai

INSTITUTION: University of Hawaii

GRANT TITLE: The biochemical and molecular mechanisms mediating recognition and specificity in a squid/luminous bacteria symbiosis

AWARD PERIOD: 1 February 1991 - 31 January 1997

OBJECTIVE: To determine the biochemical and molecular bases of host-symbiont recognition and specificity in the mutualistic association between the cephalopod *Euprymna scolopes* and its bacterial symbiont *Vibrio fischeri*. Specifically: 1) biochemically define receptor-ligand interactions between partners during the recognition process; 2) characterize those aspects of the host-created environment surrounding the symbiont that participate in specificity; and, 3) taking advantage of the ability to infect the host under experimental conditions, determine the fidelity of specificity among Indowest-Pacific and Mediterranean sepiolids and their bacterial partners.

APPROACH: We have used currently available tools of biochemistry and molecular biology to accomplish our research aims. To study receptor-ligand interactions, we have employed a wide array of cytochemical techniques coupled with confocal microscopy to determine specific types of interactions and define the precise cell types involved. In efforts to define the host-created environment, we have used 2-D gel electrophoresis to characterize the proteomes of aposymbiotic and symbiotic animals, as well as adult animals over the diel cycle. In addition, we have generated cDNA libraries of light organs that were used in this study to characterize the occurrence of a halide peroxidase in the symbiotic tissue. For comparative studies, we collected specimens from several locations. Molecular phylogenies were constructed for both the hosts and symbionts, and host habitat loyalty was assessed by determining whether strains isolated from a given host were capable of infecting other host species, both alone and in competition with native strains. In addition, using GFP-labeled bacteria in infection experiments, coupled with confocal microscopy, we characterized the mechanism by which non-native strains are outcompeted.

ACCOMPLISHMENTS: We have determined that recognition and specificity in the squid-vibrio association involves complex, redundant mechanisms of the host and symbiont. Mannose adhesin-glycan interactions are required for infection, and bacteria-induced rearrangement of host cytoskeleton results in an increasing intimacy of the bacteria-host cell-cell interaction in the days following infection. We have determined that two host cell types are interacting with the bacterial symbionts: the epithelial lining of the crypt space, and a population of macrophages that are trafficked into the crypts. By transmission electron microscopy, we have observed bacterial cells within these host macrophages. We are presently defining whether these macrophages phagocytose *V. fischeri*, or nonspecific interlopers into the light organ crypts. Two-dimensional gel electrophoresis has revealed dramatic symbiosis-induced changes in the light organs of the host, both during

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the onset of the symbiosis and during the progression of the symbiosis through its diel cycle. A halide peroxidase, related to the mammalian antimicrobial protein myeloperoxidase, occurs in high abundance in the light organ, and other tissues (accessory nidamental gland and intestine) that interact with bacteria in the squid host. The onset of symbiosis causes a lowering of the expression of this gene in the light organ. Molecular phylogenies of the partners of this symbiosis provide strong evidence for coevolution of host and symbiont. In cross-infection experiments, all squid symbionts are capable of infecting any squid host. However, the ability of a strain to compete successfully with other strains for dominance in the light organ varies; specifically, the strain that wins in a competition will always be the strain from the host most closely related to the native host. This pattern of symbiotic competence directly mirrors the molecular phylogenies. An enhanced ability to bind to host epithelia appears to be at least one mechanism by which native strains effect dominance in the light organ.

CONCLUSIONS: The *Euprymna scolopes-Vibrio fischeri* association is an excellent model for studying a variety of aspects of symbiotic associations, including specificity and recognition, development, and mechanisms by which a stable, persistent associations are created between animals and their bacterial partners.

SIGNIFICANCE: In general, these studies pioneered the development of the squid-vibrio model. Our specific findings show that: 1) multiple mechanisms are involved in the determination of specificity, including both receptor-ligand interactions and the creation of a biochemical environment in the crypt space in which only the specific symbiont can survive; 2) symbiosis drastically changes the biochemical and molecular signature of the associated tissues; 3) mechanisms thus far associated only with antimicrobial activity are involved in the modulation of a cooperative association; and, 4) determinants of coevolution between host and symbiont are expressed at the initial stages of the symbiosis and are mediated by differences in cell-surface molecules.

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